



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANT : Seung U. Kim  
SERIAL NO. : 09/887,145  
FILED : June 22, 2001  
FOR : "IMMORTALIZED HUMAN MICROGLIA CELL  
AND CONTINUOUS CELL LINE"  
EXAMINERS : Christopher J. Nichols & Gary Kunz  
GROUP ART UNIT : 1647  
ATTORNEY'S DOCKET NO. : UBC-002

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Attorney for applicant: David Prashker

Signature: David Prashker

Date: Sept. 19, 2003

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MARKED UP VERSION OF AMENDED SPECIFICATION SUBMITTED  
PURSUANT TO 37 C.F.R.1.121

Commissioner for Patents  
P.O. Box 1450  
Alexandria, Virginia 22313-1450

Sir:

Applicant, in fulfillment of and in accordance with the requirements of 37 C.R.F. 1.121(b)(1)(iii), hereby submits a marked up version of amendments to the Specification which appear at the following location:

Page 7, line 1;

Pages 27-29; and

Page 31, line 4.

Respectfully submitted,

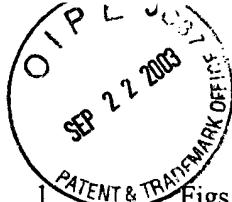
SEUNG U. KIM

Date: Sept. 19, 2003

By:



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1 Figs. 6A-6E [B] are graphs showing the results of ELISA analyses for cytokines and  
2 chemokines released from normal human microglia and HMO6 immortalized human  
3 microglia cells; and

4 Fig. 7 is a photograph shows the cytogenetic analysis of HMO6 immortalized human  
5 microglia cells as the normal karyotype of human cells.

6  
7 DETAILED DESCRIPTION OF THE INVENTION

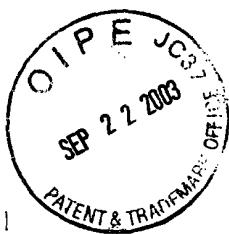
8  
9 The present invention is the establishment and characterization of several continuous  
10 cell lines of immortalized human microglia, labeled as HMO6, generated by transfection of  
11 embryonic (fetal) human microglia (HM) with a retroviral vector containing cDNA for the v-  
12 myc oncogene. The invention provides a phenotypic characterization of these immortalized  
13 human microglia; and discloses the expression of cytokines and chemokines following  
14 exposure to  $\beta$  amyloid peptides using HM and HMO6 cells. For a clearer understanding and  
15 better appreciation of the subject matter as a whole which comprises the present invention,  
16 the detailed description will be presented as separate sections.

17

18 I. A Preferred Method For Producing Immortalized Human Microglia Cells And  
19 Continuous Cell Lines  
20

21 'Human microglial cell line, as used herein, means a human-derived cell line with  
22 microglial characteristics, including at least the specific antigens CD68 and CD11b. Also, as  
23 used herein, "non-fetal" refers to the fact that the progeny cells are expanded from

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Table E1: Sequences of PCR Primers

	<u>Gene</u>	<u>Sequence</u>	<u>Product Size</u> (bp)
6	CD68 sense	AGATTCGAGTCATGTACACAACCCA [SEQ ID NO:1]	279
7	CD68 antisense	GGTGCTTGGAGATCTCGAAG [SEQ ID NO:2]	
9	P <sub>2Y1</sub> R sense	TGTGGTGTACCCCCCTCAAGTCCC [SEQ ID NO:3]	260
10	P <sub>2Y1</sub> R antisense	ATCCGTAACAGCCCAGAACATCAGCA [SEQ ID NO:4]	
12	P <sub>2Y2</sub> R sense	CCAGGCCCCGTGCTCTACTTTG [SEQ ID NO:5]	367
13	P <sub>2Y2</sub> R antisense	CATGTTGATGGCGTTGAGGGTGTG [SEQ ID NO:6]	
15	CXCR4 sense	TTCTACCCCAATGACTTGTG [SEQ ID NO:7]	206
16	CXCR4 antisense	ATGTAGTAAGGCAGCCAACA [SEQ ID NO:8]	
18	MIP-1 $\alpha$ sense	ACCATGGCTCTCTGCAACCA [SEQ ID NO:9]	393
19	MIP-1 $\alpha$ antisense	TTAAGAAGAGTCCCACAGTG [SEQ ID NO:10]	
21	MIP-1 $\beta$ sense	CCTGCTGCTTTCTTACACC [SEQ ID NO:11]	336
22	MIP-1 $\beta$ antisense	CACCTAATACAATAACACCGGC [SEQ ID NO:12]	
24	MCP-1 sense	ATAGCAGCCACCTTCATTCC [SEQ ID NO:13]	466
25	MCP-1 antisense	TTCCCCAAGTCTCTGTATCT [SEQ ID NO:14]	
27	IL-1 $\beta$ sense	AAAAGCTTGGTATGTCTGG [SEQ ID NO:15]	179
28	IL-1 $\beta$ antisense	TTTCAACACGCAGGACAGG [SEQ ID NO:16]	
30	IL-2 sense	ATGGTTGCTGTCTCATCAGC [SEQ ID NO:17]	301
31	IL-2 antisense	CTGGAGCATTACTGCTGGA [SEQ ID NO:18]	
33	IL-3 sense	ATGAGCCGCCTGCCCGTCCTG [SEQ ID NO:19]	459
34	IL-3 antisense	AAGATCGCGAGGCTAAAGTCGTCTGTTG [SEQ ID NO:20]	
36	IL-4 sense	GACACAAGTCAATATCACC [SEQ ID NO:21]	337
37	IL-4 antisense	AAGTTTCCAACGTACTCTG [SEQ ID NO:22]	
39	IL-5 sense	GAGGATGCTTCTGCATTGAGTTG [SEQ ID NO:23]	295
40	IL-5 antisense	GTCAATGTATTCCTTATTAAGGACAAG [SEQ ID NO:24]	
42	IL-6 sense	GTGTGAAAGCAGCAAAGAGGC [SEQ ID NO:25]	159
43	IL-6 antisense	CTGGAGGTACTCTAGGTATAC [SEQ ID NO:26]	

Table E1: Sequences of PCR Primers (continued)

3	4	5	Gene	Sequence	Product Size (bp)
6	7	8	IL-7 sense	TGTTGAAC TGC ACTGGCCAG [SEQ ID NO:27]	484
9	10	11	IL-7 antisense	GCAACTGATA CTTACATGG [SEQ ID NO:28]	
12	13	14	IL-8 sense	ATGACTTCCAAGCTGGCCGTG [SEQ ID NO:29]	301
15	16	17	IL-8 antisense	TATGAATTCTCAGCCCTCTTCAAAA [SEQ ID NO:30]	
18	19	20	IL-9 sense	ATGCTTCTGGCCATGGTCT [SEQ ID NO:31]	375
21	22	23	IL-9 antisense	TATCTTGCCTCTCATCCCTC [SEQ ID NO:32]	
24	25	26	IL-10 sense	AGATCTCCGAGATGCCTTCAGCAGA [SEQ ID NO:33]	194
27	28	29	IL-10 antisense	CCTTGATGTCTGGGTCTGGTTCTC [SEQ ID NO:34]	
30	31	32	IL-11 sense	ACTGCTGCTGCTGAAGACTCGGCTGTGA [SEQ ID NO:35]	295
33	34	35	IL-11 antisense	ATGGGAAAGAGCCAGGGCAGAAGTCTGT [SEQ ID NO:36]	
36	37	38	IL-12 sense	TCACAAAGGAGGCGAGGTTCTAACGC [SEQ ID NO:37]	213
39	40	41	IL-12 antisense	CCTCTGCTGCTTTGACACTGAATG [SEQ ID NO:38]	
42	43	44	IL-13 sense	ACCCAGAAC CAGAAGGCTCCG [SEQ ID NO:39]	198
45	46	47	IL-13 antisense	TCAGTTGAACCGTCCCTGGCG [SEQ ID NO:40]	
48	49	50	IL-15 sense	AAACCCCTGCCATAGCCA ACTCTT [SEQ ID NO:41]	202
51	52	53	IL-15 antisense	CTTCTGTTTAGGGAGCCCTGC ACT [SEQ ID NO:42]	
54	55	56	TNF- $\alpha$ sense	CAAAGTAGACCTGCCAGAC [SEQ ID NO:43]	490
57	58	59	TNF- $\alpha$ antisense	GACCTCTCTCTAATCAGCCC [SEQ ID NO:44]	
60	61	62	NF-M sense	TGGGAAATGGCTCGTCATT [SEQ ID NO:45]	333
63	64	65	NF-M antisense	CTTCATGGAAGCGGGCCAATT [SEQ ID NO:46]	
66	67	68	MBP sense	ACACGGGCATCCTTGACTCCATCGG [SEQ ID NO:47]	510
69	70	71	MBP antisense	TCCGGAACCAGGTGGTTTCAGCG [SEQ ID NO:48]	
72	73	74	GFAP sense	GCAGAGATGATGGAGCTCAATGACC [SEQ ID NO:49]	266
75	76	77	GFAP antisense	GTTTCATCCTGGAGCTTCTGCCTCA [SEQ ID NO:50]	
78	79	80	B7-2 sense	CTCTTGATGGCCTTCCTG [SEQ ID NO:51]	464
81	82	83	B7-2 antisense	CTTAGGTTCTGGGTAAACCGTG [SEQ ID NO:52]	

1 Table El: Sequences of PCR Primers (continued)  
2

3	<u>Gene</u>	<u>Sequence</u>	<u>Product Size</u>
4	G3PDH sense	CCATGTTCGTCATGGGTGTGAACCA [SEQ ID NO:53]	251
7	G3PDH antisense	GCCAGTAGAGGCAGGGATGATGTT [SEQ ID NO:54]	

8 bp = base pairs.  
9  
10

1 **Gene expression of cytokines and chemokines following AB treatment**  
2 Gene expression of cytokines and chemokines in HM or HM06.A1 cells was  
3 examined following a 6 hr treatment with or without 20  $\mu$ M of A $\beta$ <sub>25-35</sub> (NH<sub>2</sub>-  
4 GSNKGAIIGLM-COOH) [SED ID NO:55]. LPS at 100 ng/ml was used in microglial cultures  
5 since LPS is a potent activator of microglia [Gebicke-Jacter *J. Neurosci.* 9: 187-194 (1989);  
6 Suzumuraetal., *Brain Res.* 545: 301-306 (1991)].

7  
8        **ELISA analysis**  
9        Production of TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IL-8 or MIP-1 $\alpha$  in normal human microglial cells  
10      or HMO6.A1 cells was determined in spent culture supernatants using ELISA kits specific  
11      for human TNF- $\alpha$ , IL-1 $\beta$ , IL-6, IL-8 or MIP-1 $\alpha$  (R&D Systems, capable of detecting TNF- $\alpha$   
12      at 4.4 pg/ml, IL-1 $\beta$  at 1 pg/ml, IL-6 at 0.70 pg/ml, IL-8 at 10 pg/ml and MIP-1 $\alpha$  at 10 pg/ml).  
13      At the end of each experiment, culture supernatants were collected, centrifuged, and stored at  
14      -70°C.